Patent Office

09/719245

INVESTOR IN PEOPLE

GB 99/01848

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Signed

Dated 8 July 1999

Patents Form 1/77

Act 1977

10 JUL 1998

The Patent Office

9814888.5

9
10JUL98 E374584-7 D02684
P01/7700 25.00 - 9814888.5

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

10 JUL 1998

The Patent Office

Cardiff Road
Newport
Gwent NP9 1RH

1. Your reference

P219931/LXM/RMC

2. Patent application number
(The Patent Office will fill in this part)

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

The Queen's University of Belfast
8 Malone Road
BELFAST
BT9 5BN

Patents ADP number *(if you know it)*

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

"Peptide"

5. Name of your agent *(if you have one)*

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

Murgitroyd & Company
373 Scotland Street
GLASGOW
G5 8QA

Patents ADP number *(if you know it)*

1198013

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	7
Claim(s)	
Abstract	
Drawing(s)	3

10. If you are also filing any of the following, state how many against each item.

Priority documents	–
Translations of priority documents	–
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	–
Request for preliminary examination and search *(Patents Form 9/77)*	–
Request for substantive examination *(Patents Form 10/77)*	–
Any other documents *(please specify)*	–

11. I/We request the grant of a patent on the basis of this application.

Signature Murgitroyd & Company Date 09/07/98

Murgitroyd & Company

12. Name and daytime telephone number of person to contact in the United Kingdom: Roisin McNally 0141 307 8400

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

a) *If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.*

b) *Write your answers in capital letters using black ink or you may type them.*

c) *If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.*

d) *If you have answered 'Yes' Patents Form 7/77 will need to be filed.*

e) *Once you have filled in the form you must remember to sign and date it.*

f) *For details of the fee and ways to pay please contact the Patent Office.*

"Peptide"

The present invention relates to a modified analogue of the signal peptide sequence from Karposi syndrome fibroblast growth factor (kFGF) to be used as a cell-permeant vehicle for the intracellular delivery of covalently linked anti-sense peptide nucleic acid sequences (PNAs).

PNAs have potential uses as antisense molecules for the control of gene expression. Since they are capable of binding tightly to DNA and RNA targets thus preventing DNA transcription to RNA and RNA translation to protein. These molecules thus have two potential uses of commercial importance:

1. As research reagents where scientists use antisense strategies to ablate selected genes in order to understand their function.

2. As pharmaceutical compounds for companies seeking to develop nucleic acid-based therapies.

Conventional anti-sense oligonucletide in vivo delivery is highly inefficient, even if long-lasting, less polar

The invention provides modified peptide sequence I as detailed herein.

The invention also provides peptide sequences II and III as detailed herein.

The invention provides use of a peptide as defined herein together with lysine residues for multiple presentation of peptide nucleic acids.

The invention further provides use of peptides as defined herein together with lysine residues in the simultaneous presentation of different peptides nucleic acids.

The present invention combines the two above technologies to use CPPI to deliver PNAs to in vivo targets.

Example

In order to determine the best delivery system, a comparison of the ability of three different cell permeant peptides to accumulate in whole cells was undertaken. The three peptides (Table 1) were labelled with carboxyfluorescein and the amount accumulated intracellularly was assayed after exposure of cells to 50μM; peptide II = 0.4μM; peptide III = ~0.4μM.

Table 1

I	CFI	A A V A L L P A V L L A L L A P K K K
II	CFI	R F A R K G A L R Q K N V H E V K N
III	CFI	R P R P Q Q F O G L M

r

Key Peptide I : modified kFGF signal sequence
Peptide II : PKC pseudosubstrate sequence
Peptide III : modified substance P
CFI : Carboxyfluorescein
Or : Ornithine
Boldface : Modifications to original sequence

Peptide I was modified to contain three lysines C-terminal of the hydrophobic signal sequence. This peptide, therefore, can accommodate three PNAs, each bonded to a lysine epsilon amino group. This can be extended using the Multiple Antigen Presentation (MAP) technology to present eight (or more) PNA's on one peptide I sequence. A 'lysine tree' constructed in this way accommodates eight copies of the same PNA (see Fig 1A), thus increasing the effective concentration delivered by each CPPI. Alternatively a carrier can be constructed containing three (or more) different PNAs directed towards different sites on the same target mRNA (see Fig. 1B). This strategy has been termed 'molecular triangulation' (Branch, A.D., 1998).

Further to the sequences illustrated in Figures 1 and 2 additional tri-lysine extension by providing three positive charges, appears to aid solubility and cell permeability to allow PNA sequences to be transported. Therefore in addition to using lysine residues to attach to PNA sequences, additional tri-lysine extension is recommended. Examples of presentation peptide using the additional try-lysine is demonstrated in Figures 3b, c, d and e and in Figure 4c and d.

Lysine extensions comprising more or less than three lysine residues may also be useful to provide additional solubility and cell permeability.

The lysine extension may be provided next to a carboxyfluorescein reporter group to enhance its fluoresence.

Fig. 1A - Multiple presentation of a single PNA species

Fig.1A Multiple presentation of a single PNA species

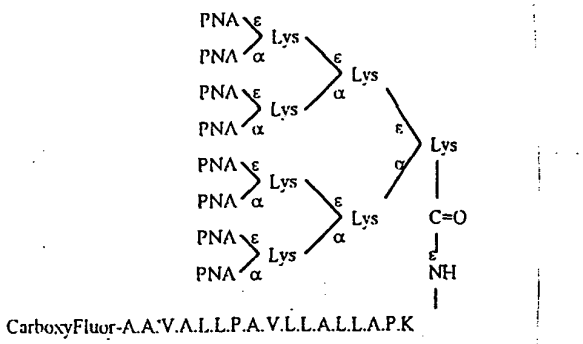
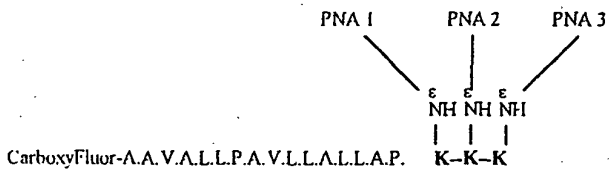


Fig. 1B - Simultaneous presentation of 3 PNAs directed to different sites on same target RNA

Fig.1B Simultaneous presentation of 3 PNAs directed to different sites on same target RNA



References

Allinquant, B., Hantraye, P., Mailleux, P., Moya, K., Bouillot, C. and Prochiantz, A (1995) Downregulation of amyloid precursor protein inhibits neurite outgrowth *in vitro* J. Cell Boil., 128: 919-927.

Branch, A.D. (1998) A good antisense molecule is hard to find. TIBS, 23: 45-50.

Jordan, S., Schwemler, C., Kosch, W., Kretschner, A., Schwenner, E., Stropp, U. and Mielke, B. (1997) Synthesis of new building blocks for peptide nucleic acids containing monomers with variations in the backbone. *Bioorg. Med. Chem. Lett.*, 7: 681-686.

Neilsen, P.E., Egholm, M., Berg, R.H. and Buchardt, O. (1991) Sequence-selective recognition of RNA by strand displacement with a thymine-substituted polyamide. *Science*, 254: 1497-1500.

Theodore, L. Derossi, D., Chassang, G., Llirbat, B., Kubes, M., Jordan, P., Chneiweiss, H., Godement, P. and Prochiantz, A. (1995) Intraneuronal delivery of protein kinase C pseudosubstrate leads to growth cone collapse. J. Neurosci., 15: 7158-7167.

Wittung, P., Kajanus, J., Edwards, K., Haaima, G., Nielson, P., Norden, B. and Malmstrom, B.G. Phospholipid membrane-permeability of peptide nucleic-acid (1995) *FEBS Lett.*, 375: 317-320.

Fig 2. Uses of modified kFGF signal peptide in Cell Permeable Peptide Import

A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P — *1a. Native kFGF signal peptide sequence*

CarboxyFluor-A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P — *1b. Signal peptide sequence with reporter group*

CarboxyFluor-A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P.K.K.K — *1c. C-terminal tri-lysine extension provides 3 positive charges aiding solubility and cell permeability*

CarboxyFluor-A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P-[PNA SEQUENCE] — *PNA forms part of the linear primary amino acid sequence*

CarboxyFluor-A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P-[PNA SEQUENCE]-K.K.K — *1d. C-terminal tri-lysine extension provides 3 positive charges aiding solubility and cell permeability*

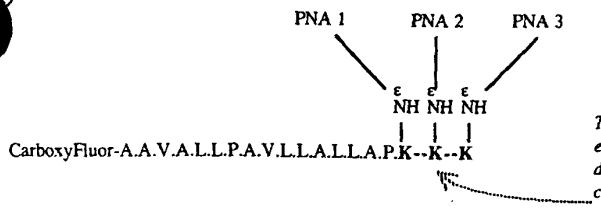
CarboxyFluor-A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P.K.K.K-[PNA SEQUENCE]

CarboxyFluor-.K.K.K---A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P-[PNA SEQUENCE] — *1e.Tri-lysyl N-terminal extension provides 3 positive charges aiding solubility and cell permeability. Proximity to the carboxyfluorescein reporter group enhances its fluoresence.*

CarboxyFluor-.K.K.K-[PNA SEQUENCE]-A.A.V.A.L.L.P.A.V.L.L.A.L.L.A.P

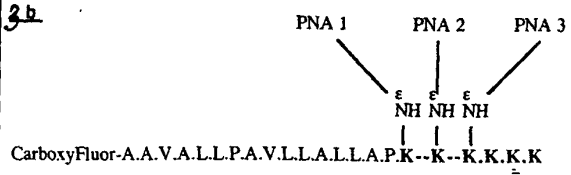
The "cargo" to be delivered intracellularly is represented as a Peptide Nucleic Acid (PNA) in Figures 1, 2 & 3. However, the various configurations of CPPI described in this patent could also be used to carry peptide sequences r oligonucleotide sequences (either native sequences r modified sequences, such as ph sphothiorates).

3a



Tri-lysyl C-terminal extension allows three different PNA sequences to be conjugated to the ε-amino groups of the lysines

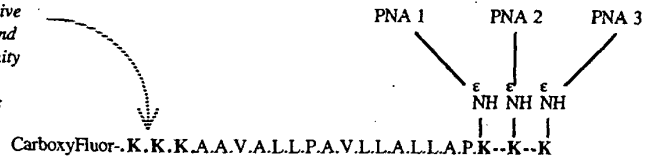
3b



Further addition of three more lysines provides 3 positive charges aiding solubility and cell permeability

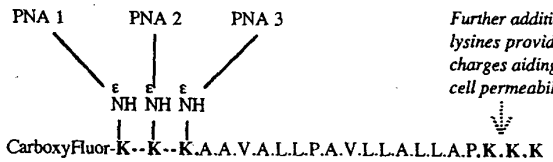
3c

Tri-lysyl N-terminal extension provides 3 positive charges aiding solubility and cell permeability. Proximity to the carboxyfluorescein reporter group enhances its fluoresence.



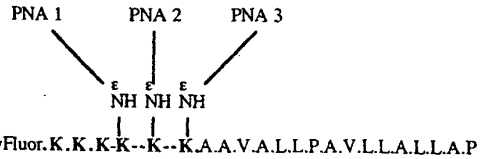
3d

N-terminal tri-lysyl extension added to kFGF signal peptide sequence allows three different PNA sequences to be conjugated to the ε-amino groups of the lysines



Further addition of three more lysines provides 3 positive charges aiding solubility and cell permeability

3e



Tri-lysyl N-terminal extension provides 3 positive charges aiding solubility and cell permeability. Proximity to the carboxyfluorescein reporter group enhances its fluoresence.